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A Review of Some Parasite Diseases of African Fish Gut Lumen Protozoa, Coccidioses, Cryptosporidium Infections, Haemoprotozoa, Haemosporidia

[1]A.Eli, [1]O.F. Briyai and [2]J.F.N. Abowei
[1]Department of Biological Science, Faculty of Science, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria
[2]Africa, Azikoro Branch, Yenagoa, Bayelsa State, Nigeria

Abstract: A review of some parasite diseases of African fish: Gut lumen protozoa, coccidioses, cryptosporidium infections, haemoprotozoa, haemosporidia was carried out from some existing literature to provide fish culturists and the public sector information on some challenges faced in culture fisheries. The Description, taxonomy, diagnosis Life cycles, biology, Epizootiology and control, Protozoans of the gut lumen, Coccidioses, Cryptosporidium Infections, Haemoprotozoa (Haemoflagellates), Haemosporidia - *Dactylosoma* and *Hemogregarines* are reviewed in this study.

Key words: African fish, coccidioses, cryptosporidium infections, gut lumen protozoa, haemoprotozoa, haemosporidia

INTRODUCTION

Infections by *Hexamita* (*Spironucleus*) are common in cultured tilapia (Landsberg, 1989) as well as in commercially reared South American cichlids in Israel, but, thus far, have not been reported from cichlids in Africa. Occasional hexamitoses occur also in farmed goldfish in Israel (Siddall and Desser, 1993). Opalinids (*Protoopalina* and *Zelleriella*) and ciliates (*Balantidium* and *Nyctotherus*) were reported from African fish of the families Schilbeidae, Mochocidae and Citharinidae (Fantham, 1918; Sandon, 1949). Amoebae have never been reported from African fish but infections have been reported in grass carp (*Ctenopharyngodon idella*) and in a neotropic catfish (*Pimelodus clarias*) and Salmonids in coldwater habitats (Sawyer *et al*., 1975). There is also an unconfirmed report on systemic amoebiasis epizooty among tilapia cultured in Auburn, Alabama, US.

Among African fish, infection by *Eimerine coccidia* has so far been demonstrated in cichlid fish, in *Clarias gariepinus*, and in eels (*Anguilla mossambica*). Other fish have not been investigated. Coccidia also infect common carp, goldfish, grass carp and silver carp. Other tropical fish found to host coccidia are farmed Gouramies (*Trichogaster trichopterus*) (Soo-Hyun and Paperna, 1993b) and, in South America, cichlids (Bekesi and Molnar, 1991; Azevedo *et al*., 1993) and characids (*Serrasalmus niger*), both with visceral coccidia (*Calyptospora* spp.) (Bassey, 2011).

Eimeria vanasi and *Goussia cichlidarum* occur in cichlid fish in Israel, Uganda and South Africa (Landsberg and Paperna, 1985, 1987). *E. vanasi* has also been recovered from *Oreochromis niloticus* introduced to Thailand (from Egypt via Japan). Carp and goldfish in Israel, as elsewhere (Kent and Hedrick, 1985), are infected by *Goussia carpelli*. A second species found on Eurasian carp, *Goussia subepithelialis* (Marincek, 1973), and *Eimeria sinensis* in silvercarp and bighead (Molnar, 1976) are absent from Israeli farmed fish. Introduced cyprinids in South Africa have not, thus far, been examined. Visceral tissue coccidioses are as yet unknown in African fish, however, liver and gonadal infections by *Calyptospora* spp. were reported from South American hosts (as well as from euryhaline killifishes in the southern USA - Overstreet *et al*., 1984). The only known coccidia from Southeast Asian tropical fish is *Goussia trichogasteri* from Gourami (Szekely and Molnar, 1992).

In Africa, *Dactylosoma* has been found in cichlids (species of *Oreochromis*, *Astatorheochromis* and *Haplochromis*) and grey mullets (Mugilidae-*Mugil cephalus*, *Liza dummerelli* and *L. richardsoni*). The latter two species of grey mullets are thus far the only known African hosts for *Hemogregarina*. Data on piscine hemogregarines and dactylosomes in Africa are scanty. Dactylosomes in cichlids (*D. mariae* - Hoare, 1930; Baker, 1960) were thus far only found in Lakes Victoria and George, with a single record from *O. mossambicus* in Transvaal, South Africa, while *Dactylosoma hannesi* and hemogregarines were only found in grey mullets from Southern Cape rivers (South Africa) (Siddall and Desser, 1992).

Cryptosporidium is a common parasite of the stomach in wild and cultured cichlid fry (*Oreochromis* spp.) in Israel (Landsberg and Paperna, 1986). Meronts

Corresponding Author: J.F.N. Abowei, Department of Biological Science, Faculty of Science, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria

and gamonts of this minute (about 5×3 μm) coccidium appear as dense spherical or mushroom-like structures located at the brush border apices of the stomaepithelium. The attached parasite is encased within the host cell wall, whose rudimentary microvilli are visible when viewed with a scanning electron microscope (Paperna,1987). Host-cell microvilli are completely lacg of other vertebrates. A further unique feature to piscine *Cryptosporidium* is the retreat of the mature zygote into the stomach mucosa or submucosa, where sporulation is completed (into eight naked sporozoites), instead of being released into the gut lumen, as occurs in the non piscine forms. Apart from cichlids, *Cryptosporidium* has been reported to infect carp and a few marine fish. It is difficult to establish whether *Cryptosporidium* is pathogenic to cichlid fry, as this infection occurs concurrently with enteric coccidiosis. Data from other infections are insufficient to be conclusive. Control methods are unknown.

In various freshwater and marine fish; in Africa: in Cichlidae, *Clarias* spp., *Bagrus* spp., Synodontidae, Mormyridae, Mugilidae and *Protopterus aethiopicus*. Trypanosomes have been reported in all major water systems of Africa (Wenyon, 1908; Hoare, 1932; Dias, 1952; Baker, 1960, 1961), with some species apparently distributed as widely as their hosts (cf. *Clarias gariepinus*). Trypanosomes occur in *C. lazera*, in the Near East, but not in cichlids or cyprinids. Trypanosomes have also been reported from introduced *Oreochromis mossambicus* in India (Mandal, 1977). Trypanosomes T. cf. mugicola (Becker and Overstreet, 1979) are widespread in grey mullet (Mugilidae) of the lagoons and rivers of southern Africa. There are no reports of vascular *Cryptobia* from Africa. This article reviews the description, taxonomy, diagnosis Life cycles, biology, Epizootiology and control, Protozoans of the gut lumen, Coccidioses, Cryptosporidium Infections, Haemoprotozoa (Haemoflagellates), Haemosporidia-*Dactylosoma* and *Hemogregarines* to enlighten fish culturists and both private/public sector some challenges likely faced in culture fisheries.

PROTOZOANS OF THE GUT LUMEN

Description, taxonomy and diagnosis: Hexamita (*Spironucleus*) [Zoomastigophorea, Diplomonadida] is 7-12 × 4-9 μm, pear shaped or round, flagellate with 3 pairs of anterior flagellae and one pair of posteriorly pointed flagellae. The taxonomic relationships (at the generic and species levels) between parasites found in different hosts are still unresolved. Examination of live flagellates in fresh smears or fixed and Giemsa stained, cannot provide sufficient details for taxonomic differentiation (Molnar, 1974).

Balantidium [Ciliophora, Vestibuliferia, Trichostomatida] is a large holociliate with a large round macronucleus, readily seen in fresh and stained bouin fixed smears (with either Giemsa or Hematoxylin) or in histological sections (Molnar and Reinhardt, 1978). Nyctotherus [Polymenophorea, Heterotrichida] are common ciliates in amphibia and have a very large cytostome. Opalines [Opalinata] are large (about 250 μm long), rounded, organisms uniformly covered with parallel rows of short flagellae. They are superficially reminiscent of ciliates, but are included among the Sarcomastigophora (flagellates) (Sandon, 1976; Foissner *et al*., 1979). They have from two to many similar nuclei and in this differ from the ciliophorans which have micro and macro nuclei. There is a difference of opinion concerning aetiological agents of visceral granuloma in tilapia and goldfish, regarded as amoeba [Sarcodina] by some (Lom and Dykova, 1992) and fungi (*Dermocystidium*-like) by others.

Plate 1 shows the Blood and gut Protozoa: a. Trypanosomes in the blood of *Liza richardsoni*, Kowei lagoon, Southeastern Cape, South Africa. b. A hemogregarine in blood of *L. richardsoni* of same locality as a. c. Light microscopic histology and electron microscopic view of a presumed hemogregarine cystozoite stages in Red sea grey mullet (*L. subviridis*). f. *Hexamita* sp. in giemsa stained smear from tilapia hybrid gut. g. *Hexamita* (*Spironucleus*) in giemsa stained smears from gut of angel fish (*Pterophyllum scalare*). h. Histological section of *Hexamita* infected tilapia hybrid.

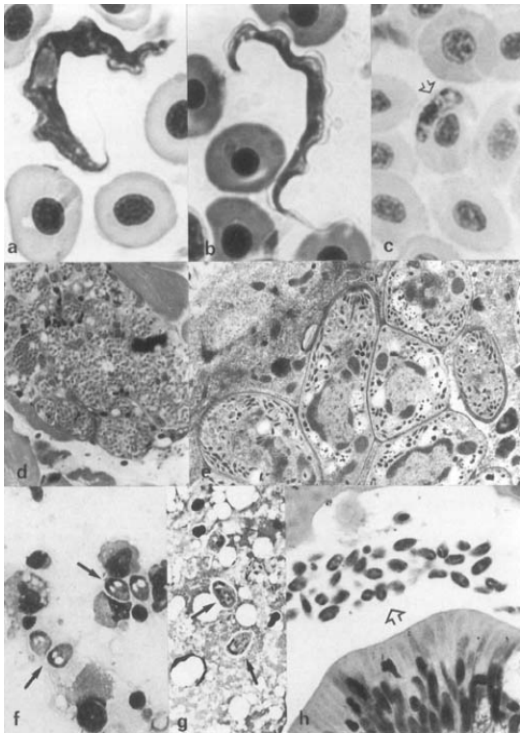

Plate 1: Blood and gut protozoa

Life cycles and biology: Both *Hexamita* and the gut ciliates divide by binary fission, it is not known if these piscine parasites may persist as resting stages in cysts. The life history of opalinids has been studied in species infecting anurans. It involves sequences of binary divisions and divisions yielding daughter cells (tomonts, which form cysts and in addition, a gamogonous process resulting in a zygocyst.

Pathology: Hexamitosis (octomitosis) in aquarium held South American cichlids, notably *Symphysodon discus* and *Pterophyllum scalare*, often coincides with poor conditions and mortalities. In recent years infection has frequently been diagnosed in tilapia hybrids cultured in Israel. Massive numbers often congest the posterior digestive tract and coincide with food retention. It is, however, not yet certain if *Hexamita* is a primary pathogen or a synergist in other clinical conditions and bacterial contaminations. Heavy infections in grass carp or South American cichlids cause haemorrhagic enteritis, with injuries to the mucosal epithelium, some necrotic changes in the liver and sometimes haemorrhagic dropsy (ascitis) (Molnar, 1974).

Heavy intestinal infection by *Cryptobia iubilans* in the South American cichlids *Herichthys cyanogutatus* and *Cichlasoma meekei* caused severe inflammation in the entire digestive tract, though the epithelial layer remained by and large intact. Oedema, atrophy and necrosis occurred in the *lamina muscularis*; lesions extended to the liver and the spleen (Dykova and Lom, 1985). Fish stopped feeding, developed dropsy, and gradually died (Bejerano, pers. comm.). A granulomatous condition in viscera associated with heavy proliferation of *Cryptobia*-like organisms has been recently found in tilapia hybrids in Israel.

Balantidiosis in grass carp causes haemorrhagic enteritis. In the terminal stage of the disease, hyperaemia and inflammation extends to the entire gut mucosa. Large numbers of parasites may accumulate in the exudate, filling the posterior end of the intestine. Histopathology reveals loss of the superficial epithelium in many places, invasion of the *lamina propria* by the parasites and multiple ulcerations (Molnar and Reihardt, 1978). Mortality of aquarium reared, South American cichlids (*Symphysodon aequifasciata*) coincided with heavy infection by opalinids (*Protoopalina symphysodontis*), resulting in congestion of the digestive tract (Foissner *et al*., 1979).

Epizootiology: There are no data on hexamitosis in Africa. Species from South American and African cichlids seem to be different, and cross infection may not be possible. Susceptibility of African cichlids to *Cryptobia iubilans* has yet to be examined; the recent occurrence of granuloma associated with a *Cryptobia*-like infection in cultured tilapia could have been the consequence of cross infection between American and African cichlids. *Balantidium ctenopharyngodonis* has not been found thus far in grass carp introduced to South Africa.

Control: There are several pharmaceutical products recommended against hexamitoses: Flagil, Enheptin, Gabbrocol (Farmitalia), Actinitrazol (Fluka). The first was used to control hexamitosis in South American cichlids. All of these are applied via medicated feeds (1–2 g/ 100 kg feeds for 3-7 days (Reichenbach-Klinke, 1980). Therapeutic control of intestinal ciliate infections has not yet been practiced.

COCCIDIOSES

Description taxonomy and diagnosisPiscine coccidia are intracellular organisms of the epithelium (of the gut, the gall bladder, the swimbladder and the kidney tubules) and tissues (liver) of epithelial origin (Bassey, 2011). Developing intracellular (endogenous) stages may be detected within their host tissues by microscopic examination of fresh tissue and stained impressions and smears (with buffered [to pH 7.2] Giemsa, after being air dried and fixed in absolute methanol). Oocysts of digestive tract-coccidia may be detected in faeces. The oocyst wall of piscine coccidia, with a few exceptions (of eels), is soft and fragile and is often lost by the end of sporulation. Therefore, if sporulation occurs prior to defecation, only the smaller naked sporocysts may be found in faeces. Coccidia are identified by the morphometry of their oocysts and sporocysts, the site of endogenous development and their position in the host cell (Dykova and Lom, 1981).

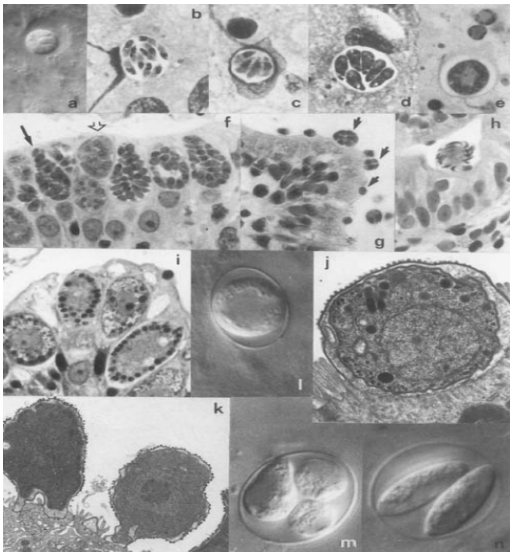

Plate 2: Intestinal coccidiosis

Plate 2 shows Intestinal Coccidiosis: a. live meront, b-e. Giemsa stained smears: b, cytoplasmic merozoites; c, intranuclear merozoites; d. formation of young macrogamonts; e, zygote with early wall formation. f–i. Histological sections: f, mature (open arrow) and divided cytoplasmic meronts (bold arrow); g. Epicytoplasmic merogony stages; h, epicytoplasmic microgamont with microgametes; i, mature macrogamonts with wall forming-like bodies (dark granules). j-k. Transmission electron microscopic view of epicytoplasmic young meront (j) and young gamonts (k). I-n. Stages from non-sporulated to sporulated oocysts (photo J.H. Landsberg).

Plate 3 shows the histology viewed with phase contrast microscopy of extraintestinal Coccidiosis: a. young gamonts (histology). b. young meronts or gamonts, Scanning Electron Microscopy (SEM). c. Dividing meront (histology). d. microgamonts (open arrow) and macrogamonts (histology). e. macrogamonts and swollen swimbladder epithelial lining (SEM). f, g, I, m, live parasites seen in pressed swimbladder between glass slides: f, macrogamonts; g, sporulating oocysts; i, oocysts with sporocysts; j, oocysts with ripe sporocysts. h, SEM of ripe oocyst, note the fragile thin oocyst wall. k. Transmission electron microscopic view of young macrogamont within a parasitophorus sac and junctions to the underlying epithelial cell (arrows). l, Tissue reaction, fibroblasts and macrophages around oocysts (arrow) regressed into the submucosal layer of the swimbladder.

Plate 4 Shows Cryptosporidiosis: a. histological section, small arrows: epicytoplasmic merogony and gamogony stages, large arrows oocyst regressed into the intestinal mucosa. b. Epicytoplasmic macrogamont, c, microgamont (arrow microgamete) and d, sporulated oocyst in the tissue view by a transmission electron microscope. e. Epicytoplasmic stages viewed by a scanning electron microscope.

The sporocyst's hard wall is either bivalved and cleaves by a longitudinal suture (in the genus *Goussia*, Paperna and Cross, 1985), or opens at the sporocyst's apex, at one pole, through a round pore (genus *Epieimeria* of eels, and some *Eimeria* s.l.) or a short apical suture. The sporocyst wall of the latter type may also form tubercules or projections and is further enclosed in a veil genus *Calyptospora* (Overstreet *et al*., 1984).

Life history and biology: Piscine coccidia develop either in the cytoplasm of the host cell or inside it's nucleus. Epicytoplasmic coccidia develop at the apex of the epithelial cell, below its brush border, bulging as they grow, together with their host cell wall, into the space about the epithelium (intestinal, swimbladder or excretory lumen) (Paperna and Cross, 1985; Molnar and Baska, 1986). Sometimes the same coccidium species has cytoplasmic, intranuclear and epicytoplasmic generations (*E. vanasi* infecting cichlid fish, Landsberg and Paperna, 1987; Soo-Hyun and Paperna, 1992). Extraintestinal coccidia apparently reach their target organ via the blood. *G. cichlidarum* also underwent endodyogenous division before becoming established in the swim-bladder

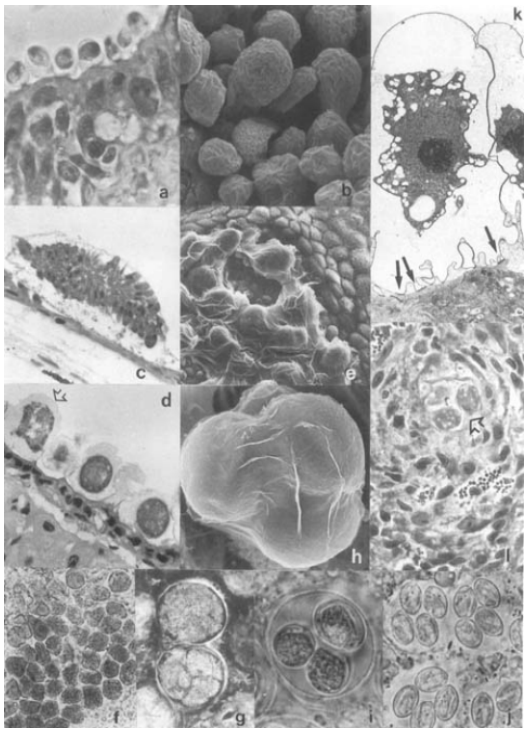

Plate 3: Extraintestinal coccidiosis

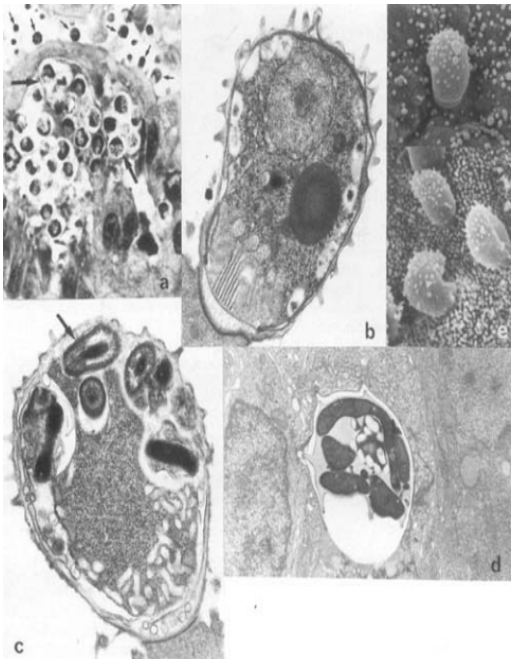

Plate 4: Cryptosporidiosi

epithelium of its cichlid host (Soo-Hyun and Paperna, 1993a).

In the epithelial cell, parasites undergo successive asexual (merogonous) divisions and a sexual process by which microgametes, differentiated from a microgamont, fuse with a macrogamont (macrogamete). The zygote thus formed becomes liberated from the host cell, while being encased in a wall. Through subsequent divisions the zygote divides into four, hard walled sporocysts, each of which further divides into two motile sporozoites. The pace of development is fast in intestinal coccidia; in *E. vanasi* 8 days from infection to sporulation (at 24-27ºC) (Soo-Hyun, 1992). In the swimbladder, coccidium (*G. cichlidarum*) endogenous development to sporozoite-containing sporocysts lasted at least 58 days at 23-26ºC (Soo-Hyun and Paperna, 1993a). These differences between intestinal and extraintestinal species are confirmed by studies of non-African species (Solangi and Overstreet, 1980; Steinhagen, 1991a).

Oocysts of digestive tract and gall bladder coccidia are evacuated with the faeces (Landsberg & Paperna, 1985, 1987; Paperna, 1990, 1991; Soo-Hyun and Paperna, 1992, 1993b). Sporulation of most gut coccidia is completed before evacuation in the faeces (endogenous sporulation). Some intestinal species, notably *G. carpelli* infecting carp and goldfish, become trapped in the gut epithelium within degenerate host cells (yellow bodies) (Kent and Hedrick, 1985). Oocysts of another carp coccidium, *G. subepithelialis*, formed in the epithelium are displaced by the regenerating epithelium into the sub-mucosal layer (Marincek, 1973; Molnar, 1984). Oocysts of the epicytoplasmic coccidium of eels *Epieimeria anguillae* also infiltrate into the mucosa rather than being evacuated into the lumen (Hine, 1975). Oocysts of visceral and internal cavity coccidia, accumulate, sporulate in the host and will be liberated only after the death of the host (Solangi and Overstreet, 1980).

Direct transmission by feeding on evacuated, sporulated oocysts has been demonstrated in several intestinal species, including those in carp *Goussia carpelli* (Steinhagen and Korting, 1988) and in cichlids *E. vanasi* (Soo-Hyun and Paperna, 1992). It has also been experimentally demonstrated that tubificid oligochaetes, of the genera *Tubifex* and *Limnodrilus*, serve as paratenic hosts: sporozoites of *G. carpelli* when ingested by the worms, excysted and invaded their gut epithelial cells. Such sporozoites remained infective, when fed with the worm, to carp 9 weeks later (Steinhagen, 1991b). Some eimerine coccidia (*Calyptospora funduli*) require an obligate intermediate host (grass shrimp) for transmission (Fournie and Overstreet, 1983). Transmission via predation and necrophagy seems to be the route of transmission not only for extraintestinal coccidia but for endogenously sporulating intestinal coccidia, while still in their host gut tissue or in the intestinal lumen (Molnar, 1984).

Pathology: The economic damage done by coccidiosis to warm water pisciculture has apparently been grossly underestimated. Since coccidiosis in fish usually manifests itself as a chronic infection, mortality is gradual and is overlooked in most farms. Losses only become evident when yields are checked at the end of the growth period. Cyprinids and cichlids contract intestinal coccidioses as soon as they hatch. Infection of *G. carpelli* has been identified in 8-day-old goldfish, with mortality occurring 30-45 days later. *G. carpelli* seems to be more pathogenic to goldfish than to carp (Kent and Hedrick, 1985). In cichlids (cultured *Oreochromis* hybrids), intestinal infection of *E. vanasi* was detected in fry by the end of nursing in their parents' mouth, losses became evident when infection reached maximum levels by two or three weeks after hatching (Soo-Hyun, 1992). Heavy infections in carp fingerlings (25-50 mm long) have been found to coincide with severe emaciation. Emaciation also occurred in infected cichlid fry. Surviving fish demonstrated spontaneous recovery, infection was low or absent in carp and goldfish or cichlids older than 2 months. Nonspecific defence response parameters (leucocytosis, eosinophylia, activation of phagocytes and elevation of natural antibody titer and of coeruloplasmin) were detected in carp infected with *G. subepithelialis* (Studnicka and Siwicki, 1990).

Damage caused by intestinal coccidioses occurs principally by the rupture of the epithelium by the escaping merozoites and oocysts (in *G. carpelli* and *E. sinensis*-Molnar, 1976, 1984; Kent and Hedrick, 1985). In the intestine of cichlid fry infected with *E. vanasi*, most damage is caused to the mucosal cells by the developing intracytoplasmic parasites (Landsberg and Paperna, 1987). Epicytoplasmic infections seem to have less effect on the gut epithelial layer and the damage induced through consumption of the nuclei by the intranuclear generations has not yet been evaluated. Inflammatory changes in intestinal coccidioses only occur following disintegration of the mucosal layer and in response to accumulated cellular debris. In nodular coccidiosis, caused in carp by *G. subepithelialis*, the accumulation of oocysts in the *lamina propria* induces inflammation with intense leucocyte infiltration (enteritis) (Marincek, 1973a; Molnar, 1984).

The *G. cichlidarum* epicytoplasmic infection leads to an intense desquamation of the swimbladder epithelial lining in cichlids (Landsberg and Paperna, 1985). Proliferation of the mucosal epithelial cells, observed in intestinal (Molnar, 1984) and in swimbladder infections, blocks the release of oocysts from the epithelium, resulting in a condition similar to that observed in nodular coccidiosis. Swim bladders of cichlids with late infections turn opaque white. The pathogenicity of the swim bladder coccidiosis to juvenile cichlids still needs to be critically evaluated.

Coccidia of *Clarias gariepinus* have not been studied. They form yellow bodies similar to those of *G. carpelli*. Data on the pathological effect of *Epieimeria anguillae* infections are available from cultured New Zealand eels (*Anguilla australis* and *A. diffenbachii*) (Hine, 1975). Oocysts which aggregate within or below the gut mucosa (in the *lamina propria*) induce inflammatory infiltration. In more severe infections, the condition is reminiscent of nodular coccidiosis, the basal membrane breaks and, following the aggregation of oocysts, the sub-mucosal tissue degrades and the loosened epithelial mucosa is sloughed off. Mature oocysts and sporocysts are passed out with necrotic tissue, eels become severely emaciated and die (Hine, 1975).

Infected *A. mossambica* in South Africa passed free sporulated oocysts, and their intestines seemed to be normal. Host response to oocyst aggregation in the livers of fish with visceral coccidioses (*Calyptospora funduli* in American killifish, *Fundulus* spp.), even when replacing up to 85% of the organ's volume, was limited to formation of a thin fibrotic capsule sometimes with collagen, or melanin (Solangi and Overstreet, 1980; Bekesi and Molnar, 1991).

Epizootiology: In Israel it would be difficult to find cichlid fry, less than 25 mm in length, of any species, wild or cultured, from earth ponds or from the hatchery, free from infection with *Eimeria vanasi*. In all these young fish, irrespective of species, intracytoplasmic, epicytoplasmic and intranuclear forms occur simultaneously. Infection declines only after the surviving fish reach 40-50 mm in length (2-3 months old). Infection in all its three forms also seems to be widespread in South African cichlids, similarly affecting all local species. Heavy, morbid infections occurred in larger fish, up to 50 mm in length (*O. mossambicus*, and *Tilapia sparmanii* during winter). Persistence, or recurrences of infection, occurred where defence responses were apparently compromised due to low temperature (<13ºC) or other stress conditions. Merogonic stages remaining inactive in on-growing fish are suspected to provide the source for renewal of infection in the new generations of fry in the following reproduction season.

Swim bladder coccidiosis infections occur only in *Oreochromis* spp. and *Sarotherodon galilaeus*. Due to the longer endogenous development, prevalence of infection extends to a wider range of age/size classes, while being absent in very young fish (less than 2 months old, (<40-50 mm long). In ponds, often all fish eventually become infected. Infection is retained in *O. mossambicus* entering marine habitats in South Africa. Coccidioses in Israeli carp and goldfish are not as frequent as in cichlids, but quantitative data on infections among fish in warm water habitats are lacking.

Control: An established protocol for chemotherapy and preventive management has not yet been formulated.

CRYPTOSPORIDIUM AND HAEMOPROTOZOA (HAEMOFLAGELLATES) INFECTIONS

Description, taxonomy and diagnosis: Piscine haemoflagellates swim freely in the blood. Members of the genus *Trypanosoma* are spindle shaped, 25-95 µm long, a single flagellum originating from a usually apical kinetoplast is connected longitudinally to the trypanosome body by an undulating membrane. The nucleus is usually single, except in the course of division and centrally positioned. *Cryptobia*, also previously called *Trypanoplasma*, are reminiscent of trypanosomes in shape, but have two flagellae connected to a single kinetoplast, one free and one tied longitudinally by an undulating membrane. Although the two are seemingly related in morphology and in means of transmission via leeches, there are sufficient biological differences to separate the two. *Cryptobia*, unlike trypanosomes, are not exclusively vascular parasites and (even sometimes the same species) also occur as ectoparasites on the fish body surface and in the digestive tract. Transmission may also be direct or by predation (Woo, 1987).

Infections in the blood are readily detected either by direct microscopic observation of fresh blood or more easily from plasma from the surface of the packed erythrocyte layer of blood, centrifuged in a heparinized haematocrit capillary. Smears prepared from whole blood or centrifuged material, after being air dried and immersed in absolute methanol, are stained in diluted Giemsa (1/10 in pH 7.0-7.4 phosphate buffer). Morphology and morphometrics are considered insufficient for specific determination of trypanosomes, and comparison of the isoenzymes of *in-vitro* cultured isolates is currently the established taxonomic methodology. This methodology is already applied to differentiate amphibian trypanosomes but thus far not to those of fish species.

Life history and biology: Trypanosome binary division is rarely observed in the blood. At the present state of knowledge it will be difficult to establish if pleomorphism, e.g., small and large forms reported in non-African species (Khan, 1976) occurs in infections of African fish. It was not evident in grey mullet infections. In the leech, ingested trypanosomes in the crop undergo several successive divisions, yielding morphologically diverse generations; amastigotes, sphaeromastigotes, and epimastigotes, which migrate to the proboscis and transform into metatrypanosomes - the infective stage, which will enter fish blood during feeding (Khan, 1976). Sphaeromastigotes and epimastigotes were revealed attached to the crop epithelial lining of an estuarine piscicolid leech (a new, yet to be described species, by

Oosthuizen, J.H., University of Pretoria, R.S.A) removed from the mouth of a trypanosome-infected grey mullet (*Mugil cephalus*; see also Chapter 17.), from Swartkop estuary, Cape region, South Africa.

Epimastigotes (Critidia) were also found in *Batrachobdelloides tricarinata*, removed from *Protopterus aethiopicus* and *Barbus altianalis* from Lake Victoria, where trypanosomes (*T. mukasai*) are common. Cichlids and *Bagrus bayad* are additional hosts. (Baker, 1960, 1961). This leech, as well as trypanosomes, frequently infect *Clarias gariepinus* in Israel. The development of *Cryptobia* in the leech does not resemble the sequential development of trypanosomes. *Cryptobia* ingested with blood becomes rounded and following binary fission apparently yields infective forms which migrate (within 72 h, compared with weeks in trypanosomes) to the proboscis. Reciprocal transformation of vascular stages into ectoparasitic forms also allows direct transmission between fish hosts (Woo, 1987).

Pathology and epizootiology: Natural infection with trypanosomes may be very common, particularly where the leech vector is also common (see Chapter 17.). In Lake Victoria, 54% of *Oreochromis variabilis* and 50% of *O. esculenta* were infected, and 20% of *Oreochromis niloticus* in Lake George (Baker, 1960, 1961). Infection in catfish (*Clarias gariepinus* and *Bagrus* spp.) also seems to be very high (in all four *Bagrus docmac* examined in L. Victoria) but quantitative data are lacking. Prevalence of infection, in Cape riverine and estuarine mullets, is about 50%, but heavy infections are rarer (about 15%). In none of the instances of natural infection was there any evidence of adverse effects on the host. Decrease in haematocrit and haemoglobin levels and evidence of accelerated haemopoiesis have been reported in some infections (by *T. murmanensis*, Khan, 1977).

In experimental infections of carp fingerlings with *T. danilewskyi*, where levels of parasitaemia reached 10^5 per mm^3, some fish develop ascites, others become oedematous; infiltrative and proliferative changes occur in the renal tissue, the pancreas and in various connective tissues (Lom *et al*., 1986). Anaemia and anorexia were reported in goldfish with high levels of parasitaemia, with the same trypanosome (Nazrul Islam and Woo, 1991, 1991a). The vector leech of *T. danilewskyi* (*Piscicola geometra*) is a cold water species (Lom, 1979), unlikely to be introduced into warm water carp rearing systems. Infections with vascular *Cryptobia* are far more pathogenic, causing anaemia, splenomegalia, exophthalmia and ascites; anorexia has also been reported. Juvenile fish, salmonids and carp, are particularly susceptible, but all cases are in coldwater culture systems (Lom *et al*., 1986; Woo, 1987).

Control: At present the only practical means which may be recommended is environmental control by elimination of leeches.

HAEMOSPORIDIA - DACTYLOSOMA AND HEMOGREGARINES: DESCRIPTION AND TAXONOMY

Parasites of the circulating erythrocyte *Dactylosoma* [syn.: *Babesiosoma, Haemohormidium*] or of both circulating erythrocytes and of the reticulo-endothelial tissues - *Haemogregarina* [and *Cyrilia* in South America]. Only asexually dividing and resting stages (merogony stages and gametocytes) occur in the piscine host, while the gamogonous process takes place in the invertebrate (vector) host, which is a leech. Infected cells are detected from blood smears and tissue imprints (touch preparations), air dried, absolute methanol fixed and Giemsa stained (same as for Haemoflagellates). Waiting comma-shaped gametocystes of *Dactylosoma* are readily confused with young stages of hemogregarines.

Fully grown hemogregarine meronts and gametocytes occupy the entire long axis of the erythrocyte, and division is longitudinal. Piscine dactylosomes divide either into 4, in cross-like, or into 8 in octagonal, formation. Dactylosomes are related to hemogregarines, and both show affinities with mammalian piroplasms (Bartha and Desser, 1989). Dactylosomids with only quadruple division were regarded as a separate genus *Babesiosoma* (Jakowska and Nigrelli, 1956) while those dividing to 8 were named *Haemohormidium* (Khan, 1980), but it appears that the same species had alternating generations forming 4 and 8 progeny (Paperna, 1981).

Plate 5 Shows Haemosporida, Myxosporea and Microsporea: A. *Dactylosoma hannesi* from *Mugil cephalus*, Kowie lagoon southeastern Cape, South Africa. *B. myxobolus* sp. from gills and dermal cysts in cichlids of Lake Victoria. *C. myxobolus* from viscera of cichlids in Southern Africa, and the East African lakes. *D. myxobolus* from gills of *Labeo* spp. from Ruaha river,

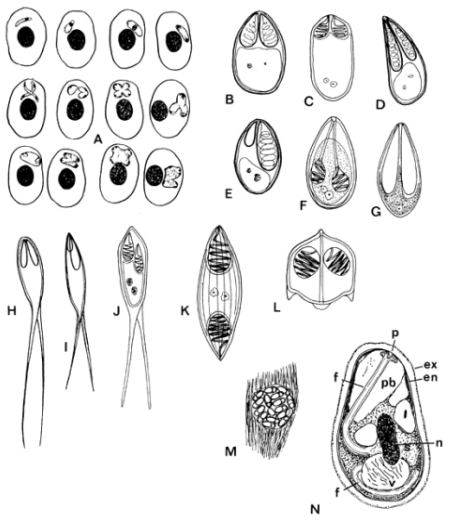

Plate 5: Haemosporida, myxosporea and microsporea

Tanzania. *E. myxobolus* sp. from gills of *Labeo senegalensis*, Volta lake. *F. myxobolus equatorialis* Landsberg (1985), from tilapia hybrids, Israel. *G. myxobolus amieti*, Fomena *et al*., 1984/1985, from *Ctenopoma nanum*, Cameroon. *H. henneguya* from gills of *Lates albertianus*, L. Albert (x). *I. henneguya* from gills of *Citharinus citharus*, Volta lake, Ghana.

Life history and biology: The definitive host and vector of both dactylosomes and hemogregarines are leeches (Lainson, 1984; Bartha and Desser, 1989; Siddall and Desser, 1992). Infection is transmitted to and from the leech during blood sucking. Ingested macrogametocytes, while becoming associated with a microgametocyte (syzygy process), penetrate the surface of the gut epithelial cell. Development of piscine dactylosomes in the leech is unknown. In the frog species, *Dactylosoma stadleri*, gametocytes are isogamous and only one microgamete is formed. The zygote penetrates the epithelial cell where it divides into 8 sporozoites. Sporozoites migrate into the salivary cells, where following a division into four (a merogony), infective stages are formed (Bartha and Desser, 1989). In piscine hemogregarines (*H. myxocephali* and *Cyrilia gomesi*) four a-flagellate microgametes are formed of which one fertilises the macrogametocyte.

In piscine hemogregarines, the zygote undergoes multiple divisions producing 16-32 sporozoites (Lainson, 1984; Siddall and Desser, 1992). Released sporozoites reinfect the epithelial fspring of this division migrate into the salivary cells connected to the proboscis (Siddall and Desser, 1993). In the fish host, merogonous division of dactylosomes occurs only in erythrocytes. Piscine hemogregarines divide in the erythrocytes, while some (such as *H. simondi* in *Solea solea*, Kirmse, 1979) will also have a pre-erythrocytic merogony in circulating and tissue leucocytes (macrophages). Furthermore, large cyst-like bodies, containing numerous hemogregarine merozoites, were reported in the visceral organs and muscles of several, thus far only marine, fish (Furguson and Roberts, 1975; Paperna, 1979; Paperna and Sabnai, 1982). It is not known if these resting stages are transmissible through predation.

Pathology and epizootiology: The only pathologically significant infections are the leucocytic hemogregarines which induce proliferative lesions, thus far reported only from cultured marine fish. Lesions may be comprised either entirely of encapsulated aggregates of merozoites (Paperna, 1979) or of infected macrophages embedded in granulomatous tissue (Furguson and Roberts, 1975). Incidence of infection by *D. mariae* in L. Victoria, in *O. esculenta* is 46% and in *O. variabilis* 70%. *D. hannesi* infection in grey mullets of the Cape coastal rivers is sporadic, only occurring in one fish species (*M. cephalus*) where the level of parasitaemia reached 3.2% and revealed dividing stages. In the remaining fish, parasitaemia was below 0.06%, and infection was comprised predominantly of non-dividing stages. Infection by hemogregarines in the same fish was rare.

CONCLUSION

Protozoans of the gut lumen, Coccidioses, Cryptosporidium Infections, Haemoprotozoa (Haemoflagellates), Haemosporidia - *Dactylosoma* and *Hemogregarines* are some parasite diseases fish culturists and both private/public sector need to know as some challenges likely faced in culture fisheries.

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